BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Jan; 18 (1): 63 - 66

Inhibition of β-myosin heavy chain gene expression in pressure overload rat heart by losartan and captopril[1]

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KEY WORDS renin-angiotensin system; losartan; captopril; aortic coarctation; heart hypertrophy; myosin; Northern blotting; gene expression regulation; hemodynamics

AIM: To study the effects of losartan and captopril on β-myosin heavy chain (MHC), and α-MHC gene expression. **METHODS**: Pressure overload was produced by abdominal aortic coarctation (AAC) in rats. α-and β-MHC mRNA were measured by Northern blot. **RESULTS**: In left ventricular myocardium of sham-operated rats, the α-MHC mRNA predominated, while the β-MHC mRNA was only detectable. In response AAC, there was a 70-fold increase in the β-MHC mRNA ($P<0.01$), while α-MHC mRNA reduced to 26% ($P<0.01$). Losartan (3 mg·kg^{-1}·d^{-1}, ig for 11 d) to AAC rats caused inhibitions of β-MHC by 96 % and α-MHC by 86 % gene expression without lowering blood pressure. A reduction in β-MHC mRNA was also seen in captopril-treated rats (30 mg·kg^{-1}·d^{-1}, ig for 11 d), but the inhibitory effect of captopril on α-MHC mRNA was less than that of losartan (44 % *vs* 86 %, $P<0.05$). **CONCLUSION**: The shift of MHC isoform induced by pressure overload is due to up-regulation of β-MHC and down-regulation of α-MHC gene expression. Inhibition of β-MHC gene expression by losartan is achieved primarily by direct blockade of angiotensin II type I receptors in the myocardium, independent on hemodynamics.

Myosin heavy chain (MHC), belongs to a multigene family which exists at least 2 isoforms in myocardium, α- and β-MHC. The proteins encoded by these genes associate in pairs with identical light chains to form 3 myosin isoenzymes $V_1(\alpha\alpha)$, $V_2(\alpha\beta)$, and $V_3(\beta\beta)$. Their relative proportion V_1/V_3 is decisive for ATPase activity[1,2].

In response to pressure overload, myocardium exhibits a complex molecular changes, which are not only a quantitative phenomenon that results in an increase in heart mass, but more importantly, in qualitative changes in its essential constituents. It was characterized by selective re-expression of a fetal/neonatal phenotype, such as β-MHC, α-actin[3]. But the factors and mechanisms involved in these complex alterations in cardiac MHC gene expression were still unknown. there was a significant shift of V_1 to V_3 during the development of cardiac hypertrophy in renovascular hypertensive rat heart[4]. Losartan, an angiotensin II selective receptor antagonist and captopril, an angiotensin-converting enzyme inhibitor, reversed heart hypertrophy and normalized V_1/V_3, concomitant with a lowering of blood pressure. However, it is uncertain whether the shift of cardiac myosin isoenzymes during heart hypertrophy was associated with intrinsic factors or hemodynamic load. This study investigated whether renin-angiotensin system (RAS) was involved in the modulation of MHC gene expression during cardiac hypertrophy, which subtype of angiotensin receptors contributed to this action, and whether the effects of losartan and captopril on MHC gene expression were carried on through hemodynamic mechanism.

MATERIALS AND METHODS

Abdominal aorta coarctation (AAC) Wistar ♂ rats (n = 60), 6 - 8 wk old and weighing 158 ± s 18 g, were operated under sodium pentobarbital 40 mg·kg^{-1}, ip anesthesia. A blunted 22-gauge needle was placed adjacent to the abdominal aorta proximal to the renal bifurcations just, and a ligature was made. The blunt needle was then withdrawn.

Rats were divided into 4 groups. ① AAC rats were given ig losartan 3 mg·kg^{-1}·d^{-1}; ② AAC rats were given ig captopril 30 mg·kg^{-1} *bid*; ③ AAC rats were given water ig

[1] Project supported by the Science Foundation of the Ministry of Public Health of China, № 94-1-122.
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Received 1995-11-20 Accepted 1996-09-14

once a day; ④ sham-operated rats were given water ig. All treatments were initiated on the d 2 after operation, and continued for 11 d. Tail-cuff blood pressure was monitored[4], and carotid arterial blood pressure was measured on 3, 7, and 12 d after surgery using a pressure transducer coupled to a polygraph recorder (Nihon Kohden, Japan). Finally the right ventricular free wall was separated. Left ventricular (including interventricular septum) samples were blotted dry, frozen in liquid nitrogen, and subjected to MHC messenger RNA analysis.

RNA isolation Total RNA was isolated from left ventricular tissue[5].

Northern blot hybridization The RNA 30 μg per lane was denatured with 50 % formamide/17.5 % formaldelyde, and electrophoresed on 1 % (vol/wt) agarose-formaldehyde gel, and transferred to Genescreen membrane (DuPont). The membrane was prehybridized in a solution containing 50 % formamide, 5×Denhardt's solution, 5×SSPE (containing NaCl 0.75 mol·L^{-1}, sodium phosphate 50 mmol·L^{-1}, edetic acid 5 mmol·L^{-1}), 0.5 % sodium dodecyl sulfate (SDS), and denatured salmon sperm DNA 30 mg·L^{-1} at 42 ℃ for 2 h. Hybridization was carried out in a hybridization oven (Cambridge, USA) with radiolabeled pecific [^{32}P]-oligonucleotide probe at 42 ℃ overnight. Following hybridization, the filter was washed in 2×SSPE/.1 % SDS for 10 min at room temperature, and then in .×EEPE/0.5 % SDS at 58 ℃ for 5 min. The filter was .posed to Kodak Omit X-ray film at -70 ℃ between 2 intensifier screens. Hybridization signals for each of the blot were quantified by densitometry (CS-930, Japan). All blots were normalized to the signal obtained with an oligonucleotide probe specific for the 18S ribosomal RNA.

All oligonucleotide probes were synthesized and purified by the Department of Biochemistry, University of Cincinnati, USA. The MHC oligonucleotide probes were 24 base fragments specific for unique regions in the untranslated regions of the rat α-MHC and β-MHC genes[6], which were constructed with the following sequence: 5′ CAA CGG CGA GGC TCT TTC TGC TGG 3′; 5′ CTC CAG GTC TCA GGG CTT CAC AGG 3′, respectively. The sequence of 18S rRNA was 5′ ACG GTA TCT GAT CGT CTT CGA ACC 3′, used as an internal control to normalize all data.

Statistical analysis Results are expressed as $\bar{x} \pm s$. Differences between groups were evaluated by one-way ANOVA.

RESULTS

Hemodynamics In AAC rats, systolic blood pressure was elevated beginning 3 d after operation. Coarctation of the abdominal aorta resulted in a significant difference in systolic blood pressure between the carotid and coccygeal arteries. The coccygeal systolic arterial pressure was about 20 − 30 % lower than that of carotid blood pressure in AAC rats (21.2 ± 0.7 *vs* 24.4 ± 1.0 kPa, $P < 0.05$). Both losartan and captopril treatment slightly lowered tail artery systolic pressure, but had no effect on carotid blood pressure (Tab 1).

Heart hypertrophy The left ventricular weight (LVW)/body weight (BW) ratio was determined after 12 d of AAC. Pressure overload induced an increase of cardiac mass. LVW was increased by 45 %, and resulted in an increased LVW/BW. Treatment with losartan and captopril prevented the increase in the cardiac mass by 58.3 % and 62.5 %, respectively. The LVW/BW in losartan and captopril treated rats were lower than that of ACC rats, but still higher *vs* sham rats

Tab 1. Effect of losartan ig 3 mg·kg^{-1}·d^{-1}, and captopril ig 30 mg·kg^{-1}·d^{-1}, for 11 d on arterial systolic blood pressure and cardiac mass. $n = 3-5$, $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* **Sham group.** $^{d}P > 0.05$, $^{e}P < 0.05$ *vs* **AAC.**

	Sham	AAC	Los	Cap
Carotid arterial pressure/kPa				
3 d	16.3 ± 0.8	24.3 ± 1.0^{c}	24.4 ± 0.9^{cd}	24.0 ± 1.7^{cd}
7 d	15.7 ± 1.6	26.5 ± 1.6^{c}	25.9 ± 2.7^{cd}	26.1 ± 1.5^{cd}
12 d	15.6 ± 1.3	26.7 ± 1.5^{c}	25.6 ± 2.7^{cd}	25.3 ± 1.5^{cd}
Coccygeal arterial pressure/kPa				
3 d	16.7 ± 1.3	21.2 ± 0.7^{b}	17.3 ± 0.7^{ae}	17.5 ± 2.3^{ae}
7 d	16.0 ± 2.0	21.6 ± 1.0^{b}	20.7 ± 1.7^{bd}	20.7 ± 1.7^{bd}
12 d	16.0 ± 1.7	21.3 ± 2.1^{b}	21.6 ± 1.2^{bd}	21.3 ± 1.2^{bd}
BW/g	150 ± 19	154 ± 23	148 ± 23	152 ± 20
LVW/g	0.51 ± 0.06	0.75 ± 0.11^{c}	0.61 ± 0.08^{bc}	0.60 ± 0.09^{bc}
LVW/BW, mg·kg^{-1}	3.38 ± 0.17	4.86 ± 0.18^{c}	4.12 ± 0.19^{bc}	3.92 ± 0.23^{bc}

AAC: abdominal aorta coarctated rats; BW: rat body weight; Cap: captopril-treated rats; Los: losartan-treated rats; LVW: left ventricular weight; Sham: sham-operated rats.

(Tab 1).

Expression of MHC gene The 18S rRNA as an invariant fraction of total cellular RNA was used to normalize MHC mRNA levels between samples and between groups. In sham-operated rats, the α-MHC mRNA predominated, while the β-MHC mRNA level was only detectable. In response to pressure overload, there was a 70-fold increase in β-MHC mRMA, but α-MHC mRNA only reduced to 26 % of sham-operated rats and resulting in a marked shift of α-MHC to β-MHC on 12 d after operation. Administration of sub-hypotensive dose of losartan to AAC rats caused an inhibition of β-MHC by 96 % and α-MHC by 86 % gene expression. A similar reduction in β-MHC mRNA was seen in sub-hypotensive dose of captopril treated rats, but its inhibitory effect on α-MHC mRNA was less than that of losartan (44 % vs 86 %, $P < 0.05$) (Fig 1).

DISCUSSION

Our previously study showed that the left ventricular hypertrophy induced by pressure overload in mammalian heart is accompanied by a shift in the myosin from $V_1(\alpha\alpha)$ to $V_3(\beta\beta)$[4], and the blockade of RAS with ACEI captopril and AT_1 receptor antagonist losartan reversed these changes with lowering blood pressure. In this study, the role of RAS and its blockers at a sub-hypotensive dose in regulation MHC gene expression during the development and regression of cardiac hypertrophy in pressure overloaded rats was first demonstrated.

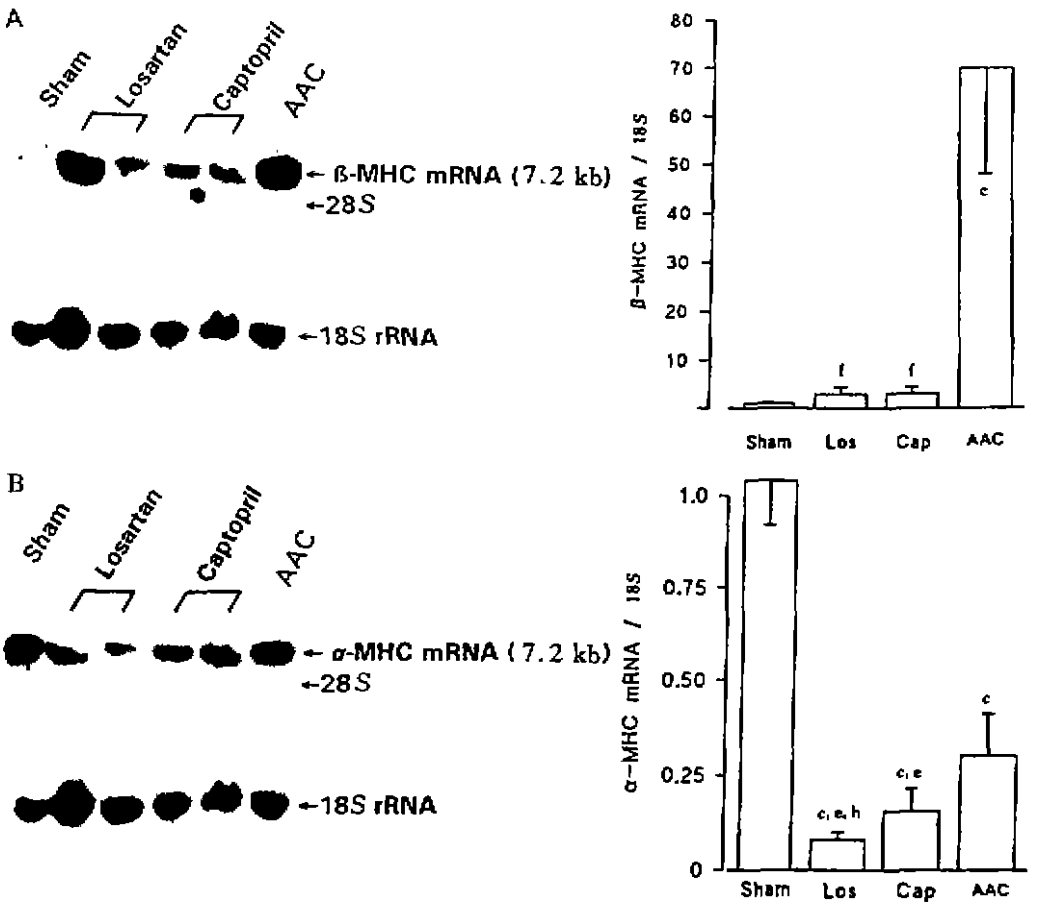

Fig 1. Autoradiography (left) and bar graph (right) showing β-MHC (A) and α-MHC (B) mRNA levels in the sham, AAC, losartan (Los, 3 mg·kg^{-1}·d^{-1}) and captopril (Cap 30 mg·kg^{-1}·d^{-1}) treated rats. Values are $\bar{x} \pm s$ for 3 to 4 samples per group. The β- or α-MHC mRNA values of the sham group was arbitrarily set at 1.0 and the remaining group were adjusted correspondingly. $^cP<0.01$ vs Sham. $^eP<0.05$, $^fP<0.01$ vs AAC; $^hP<0.05$ vs Cap.

Results showed that the shift of V_1 to V_3 occurred during heart hypertrophy in pressure overloaded rat hearts was mainly due to β-MHC gene expression up-regulation. Losartan inhibited β-MHC gene over-expression without alteration of blood pressure in AAC rats, suggested that the transcriptional activation of the β-MHC was conducted by hemodynamic independent mechanism, and not due to elevated wall stress and stretch alone.

The response between α-MHC and β-MHC gene expression during blockade of RAS with losartan and captopril in pressure overload rat is different. The fact that a selective inhibition in β-MHC transcript level without reciprocal increase in α-MHC mRNA levels, suggested that the putative pressure overload signals differ in their effects on these two genes. AAC in rats produced an extra mechanical load is often accompanied by the increase in catecholamine, Ang II, and endothelin, which may play additional role in regulating the MHC expression[6-8]. It had been shown that β-MHC gene expression was associated with activation of PKC in cultured myocytes[6,9]. Morano *et al*[10] demonstrated that the decreased α-MHC expression upon hypertension induced heart hypertrophy could be mediated via decrease of adenylate cyclase activity and thus decreased intracellular cAMP production. Combining to the present data, it most likely implied that the induction of β-MHC gene was associated with PKC activation, regulated by RAS via Ang II binding to AT_1 receptors, and modulation of α-MHC gene appeared to associate with cAMP accumulation, linked to hemodynamic stress and other factors. Further experiments are undertaken to elucidate their underlying mechanism.

REFERENCES

1 Lompre AM, Nadal-Ginard B, Mahdavi V. Expression of the cardiac ventricular α- and β-myosin heavy chain genes is developmentally and hormonally regulated. J Biol Chem 1984; **259**: 6437-46.

2 Dorn GW II, Robbins J, Ball N, Walsh RA. Myosin heavy chain regulation and myocyte contractile depression after LV hypertrophy in aortic-banded mice. Am J Physiol 1994; **267** (Pt 2): H400-H405.

3 Boheler KR, Schwartz K. Gene expression in cardiac hypertrophy. Trends Cardiovasc Med 1992; **2**: 176-82.

4 Ling Q, Guo ZG, Su Z, Guo X. Regression of cardiac hypertrophy and myosin isoenzyme patterns by losartan and captopril in renovascular hypertensive rats. Acta Pharmacol Sin 1994; **15**: 206-10.

5 Chen TH, Ling Qi, Guo ZG. Rapid one-step method for isolation of total RNA from rat myocardial tissue. Bull Hunan Med Univ 1996; **21**: 14-16.

6 Waspe LE, Ordahl CP, Simpson PC. The cardiac β-myosin heavy chain isogene is induced selectively in α_1-adrenergic receptor-stimulated hypertrophy of cultured rat heart myocytes. J Clin Invest 1990; **85**: 1206-14.

7 Parker TG, Packer SE, Schneider MD. Peptide growth factors can provoke "fetal" contractile protein gene expression in rat cardiac myocytes. J Clin Invest 1990; **85**: 507-14.

8 Wang DL, Chen JJ, Shin NL, Kao YC, Hsu KH, Huang WY, *et al*. Endothelin stimulates cardiac α- and β-myosin heavy chain gene expression. Biochem Biophys Res Commun 1992; **183**: 1260-5.

9 Kariya K, Karns LR, Simpson PC. Expression of a constitutively activated mutant of the β-isozyme of protein kinase C in cardiac myocytes stimulates the promoter of the β-myosin heavy chain isogene. J Biol Chem 1991; **266**: 10023-6.

10 Morano I, Adler K, Weismann K, Knorr A, Erdmann E, Böhm M. Correlation of myosin heavy chain expression in the rat with cAMP in different models of hypertension-induced cardiac hypertrophy. J Mol Cell Cardiol 1993; **25**: 387-94.

氯沙坦和卡托普利抑制压力超负荷大鼠心肌β-肌球蛋白重链基因表达[1]

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关键词 肾素血管紧张素系统; 氯沙坦; 卡托普利; 主动脉狭窄; 心脏肥大; 肌球蛋白; RNA印迹; 基因表达调节; 血液动力学

目的: 观察亚降压量氯沙坦和卡托普利对压力超荷大鼠心肌肌球蛋白重链(MHC)基因表达的影响. **方法**: 狭窄腹主动脉(AAC)造成心脏压力超荷, 以 18S rRNA 作内标, Northern 杂交检测 MHC mRNA. **结果**: AAC术后 12 d, 左室心肌β-MHC表达上调 70 倍, α-MHC 表达降低仅26 %. 术后 d 2 给予亚降压量氯沙坦(3 mg·kg^{-1}·d^{-1}, ig)和卡托普利(30 mg·kg^{-1}·d^{-1}, ig)治疗 11 天, 能抑制 CoA 心肌 β-MHC 基因过量表达96 %, 但不增强 α-MHC 表达. **结论**: 压力超荷导致 β-MHC 表达上调. 氯沙坦抑制 β-MHC 表达与其直接阻滞心肌 AT_1 受体有关, 而不依赖血液动力学机制.